Original Article

Ficus Bark Extract Enhances the Antihyperglycemic Activity of Glibenclamide in Rats: A Herb-Drug Interaction Study

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ABSTRACT

Objectives: The present study was undertaken to determine the interaction of glibenclamide with methanolic extract of bark of *Ficus glomerata* Roxb. (FBME).
Methods: The interaction was evaluated in normal and alloxan-induced diabetic rats using the parameters such as glucose tolerance test, acute and subacute levels of antidiabetic study and body weight estimations at various intervals. Glibenclamide was administered orally at two different doses of 300 µg/kg (low dose) and 600 µg/kg (high dose) and FBME was co-administered at the dose of 200 mg/kg. Individual treatments of above drugs were also carried out.
Results: The combination therapy of glibenclamide and FBME showed significant ($P<0.01$) hypoglycemic effect compared to their individual treatments in the parameters studied and it also showed more percentage increase in body weight than the individual treatments.
Conclusion: The present herb-drug interaction was found to be beneficial as it was observed that FBME showed synergistic effect with glibenclamide. This could provide an opportunity to reduce the dose of glibenclamide to achieve an enhanced therapeutic effect with minimal side effects.

Keywords: *Ficus glomerata,* herb-drug interaction, glibenclamide, hypoglycemic effect.

INTRODUCTION

Diabetes is the world's largest endocrine disorder characterized by dysregulation in carbohydrate, protein and fat metabolisms due to defects in insulin secretion and/or insulin action.[1] Diabetes may lead to long-term complications

including retinopathy, nephropathy, neuropathy and several others.[2] Currently, 170 million people are affected by diabetes mellitus worldwide and it is estimated that by 2025 in India about 57 million people will be affected by the disease making the country the world's highest number of diabetics.[3]

Currently available synthetic oral hypoglycemic agents like sulfonylureas, biguanides, etc. and insulin are associated with many side effects on prolonged administration.[4] A wide number of traditional medicinal plants have been used to treat diabetes mellitus in the countries like India and China. They are used as health supplements in developed countries like USA. Varieties of ingredients present in medicinal plants have potential to impart therapeutic effect in diabetes by different mechanisms.

Herbal medicines treated alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern synthetic medicines alone fail.[5] This may sometimes lead to adverse herb-drug interactions due to the reason that the multiple ingredients of medicinal plants may react with allopathic medicines.[6]

Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Synergistic therapeutic effects may complicate the dosing of long-term medicines. It has been reported that traditionally used herbs may cause hypoglycemic shock, if taken in combination with conventional drugs.[7]

Ficus glomerata Roxb. (Syn. *Ficus racemosa*) family: Moraceae, commonly known as, "Cluster fig", an evergreen tree of 15-18 m height with aerial roots, glabrous and pubescent young shoots, ovate-oblong leaves and reddish, obovoid figs.[8] Literature survey shows presence of lupeol, β-sitosterol glucoside, stigmasterol, leucoanthocynin, tannin and wax. The plant has been reported to contain a glycoside, tetracyclic triterpene-glaunol.[9] Fruit has been reported to contain glaunol, β-sitosterol, glucose, lupeol, friedelin and other phytosterols.[10] Traditional system of medicine claims the use of various parts of the plant, viz. – bark, root, leaves, fruits and latex in dysentery, diarrhea, diabetes, bilious affections, stomach ache, menorrhagea, haemoptysis and piles. The infusion of bark and leaves claims to be used as mouth wash to spongy gums and internally for effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis.[11, 12] Methanolic extract of stem bark has been reported to have the glucose lowering effect at the doses of 200 and 400 mg/kg p.o. in normal and alloxan-induced diabetic rats.[13] β-sitosterol isolated from stem bark has also been reported to possess potent hypoglycemic activity when compared to other isolated compounds.[14]

In the context of the above observations the present study was undertaken to find out the influence of methanolic extract of stem bark of *Ficus glomerata* Roxb. on antihyperglycemic activity glibenclamide at two different doses and also to establish the safety of the combination treatment.

MATERIALS AND METHODS

Extraction of plant material

Ficus glomerata Roxb. bark was collected from local areas of North Karnataka and a voucher specimen has been deposited at the departmental herbarium (GUG/BOT/Herbarium/2008-09/09). The bark of the plant was dried and pulverized to particle size (#) 40 and then was first defatted with petroleum ether (40-60^{0}C) and extracted with methanol by continuous hot percolation method using Soxhlet apparatus

at 40^0C for 48 h to obtain methanolic extract of bark of the plant. The filtrate of the extract was concentrated to dryness at 40^0c under reduced pressure in a rota flash evaporator. The yield of the methanolic extract of bark was 37.76 g (22.19 % w/w).

Experimental animals

Swiss albino mice and rats of either sex, weighing 25-30g and 150-200g respectively housed in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water *ad libitum*. (IAEC Ref. No. HKECOP/IAEC/45/2011-12).

Acute Toxicity Studies

Acute toxicity studies were conducted as per OECD guideline by 425 method (#26). The animals did not show any mortality at the dose of 2000 mg/kg and hence its $1/10^{th}$ dose i.e. 200 mg/kg was used as the therapeutic dose for the methanolic extract of the study.

Test Samples

Weighed quantities of test extracts were suspended in 1% W/V sodium carboxy methyl cellulose to prepare a suitable dosage form (Suspension). The control animals were given an equivalent volume of sodium CMC vehicle.

Drugs

Glibenclamide (Yashica Pharmaceutical Pvt. Ltd., Thane, India) and Alloxan (Prachi Enterprises, Pune, India)

Methods[15,16]

Oral glucose tolerance test

Overnight fasted rats were divided into six groups of six in each. The rats were administered orally with the respective treatment as follows.

Group I – Normal Control – equal volume of vehicle (1% W/V Sodium CMC).
Group II – Glibenclamide low dose – 300µg/kg
Group III – Glibenclamide high dose – 600µg/kg
Group IV – *Ficus glomerata* bark methanolic extract (FBME) – 200mg/kg
Group V – FBME – 200mg/kg and Glibenclamide low dose – 300µg/kg
Group VI – FBME – 200mg/kg and Glibenclamide high dose – 600µg/kg

After 30 min of the respective administration, the rats of all the groups were orally treated with 2g/kg of glucose. Blood samples were collected from tail vein just prior to glucose administration and at 30, 60 and 90 min after glucose loading. Blood glucose levels were measured immediately by using glucometer (One-Touch Horizon).

Alloxan-induced diabetic model

Diabetes mellitus was induced by intra-peritoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg) dissolved in physiological saline in overnight fasted rats. After 1 h of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 48 h blood glucose was measured by glucometer. Threshold value of blood glucose was taken between 250–300 mg/dl. One group (Group I) served as normal control, which received vehicle alone. The diabetic animals were grouped and received the following treatment for 21 days.

Group II – Diabetic control - equal volume of vehicle (1% W/V Sodium CMC).
Group III – Glibenclamide low dose – 300µg/kg
Group IV – Glibenclamide high dose – 600µg/kg

Group V – *Ficus glomerata* bark methanolic extract (FBME) – 200mg/kg
Group VI – FBME – 200mg/kg and Glibenclamide low dose – 300µg/kg
Group VII – FBME – 200mg/kg and Glibenclamide high dose – 600µg/kg

The acute study involved measuring the blood glucose levels on 1st day at 0, 1, 3 and 5 h after administration of respective treatment.

The sub-acute study involved measuring the blood glucose levels on 0, 7, 14 and 21 days, after 1 h administration of FBME and glibenclamide on respective days.

Body weight determination

Weight of rats was recorded on 0, 7, 14 and 21st days during the study period of 21 days. Mean change in body weight was calculated and tabulated.

Statistical analysis

Data were expressed as mean ± SEM and differences between the groups were statistically determined by analysis of variance (ANOVA) followed by Dunnet's test. p-values <0.5, <0.01 and <0.001 were considered as statistically significant.

RESULT AND DISCUSSION

Oral glucose tolerance test

The effect of herb-drug combinations on glucose tolerance test in normal rats is shown in Table 1. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from the initial value at 0 min and then subsequently decreased at 60 and 90 min. The combination therapy of glibenclamide with FBME showed more significant ($p<0.001$) reduction in blood glucose level compared to the individual treatment of FBME and glibenclamide low and high doses.

Alloxan-induced diabetic model

Acute study

The acute studies of FBME and both the doses of glibenclamide at 1, 3 and 5 h after the dose administration reduced the alloxan-induced sugar level very significantly ($p<0.001$) compared to the individual treatment of FBME and glibenclamide low and high doses (Table 2).

Sub-acute study

The sub-acute studies of FBME and both the doses of glibenclamide on 7th, 14th and 21st day of treatment showed significant ($p<0.01$) reduction of elevated blood glucose compared to the individual treatment of FBME and glibenclamide in both the doses. It was observed that the combination therapy brought back the alloxan-induced diabetes to normal state after 21 days treatment, which was evidenced by higher percentage reduction in blood glucose by the combination therpy. Glibenclamide at low and high doses showed dose dependent anti-hyperglycemic activity. However FBME – 200mg/kg individual treatment was able to reduce the elevated blood glucose level significantly but to a lesser extent than the combination therapy. On the 21st day percentage reduction in blood glucose level of low dose glibenclamide, high dose glibenclamide and FBME were 69.05%, 72.03% and 64.55% respectively. While the combination of low dose glibenclamide with FBME and high dose glibenclamide with FBME were 75.00% and 78.55% respectively. The results revealed that the highest percentage reduction in blood glucose level in alloxan-induced diabetic rats was exhibited by the high dose glibenclamide with FBME (Table 3).

Body weight determination

The body weight of diabetic control rats was decreased by 29.3% during the period of study. Combination therapy of glibenclamide both doses with FBME not only prevented weight loss of diabetic rats but also brought a gradual increase in weight compared to the individual treatment of PBME and glibenclamide both doses (Table 4).

Alloxan-induced hyperglycemia has been described as one of the experimental methods to study the activity of hypoglycemic agents. Alloxan, a β-cytotoxin causes a massive destruction of β-cells of islets of Langerhans, resulting in reduced synthesis and release of insulin. The function of the insulin system is suppressed leading to hyperglycemia. Alloxan-induced diabetes is characterized by loss in body weight and increased food intake. Body weight loss might be the result of protein wasting due to defect in carbohydrate metabolism and excessive breakdown of tissue protein.[17]

Oral administration of FBME, both doses of glibenclamide and the combination therapy of the above have shown significant ($p<0.01$) reduction in blood glucose level at 60 and 90 min after glucose load. The reduction in blood glucose level was more significant ($p<0.001$) with the combination therapy than the single treatment. This suggests that the administration of FBME with low and high doses of glibenclamide can more significantly reduce the postprandial hyperglycemia.

The acute and sub-acute studies of the single treatment of FBME, both doses of glibenclamide and the combination therapy of the above have shown significant decrease in glycemia. The percentage reduction glycemia after 21 days treatment was more with combination therapy than single treatment suggesting the advantage of combination in long term treatment.

Oral daily administration of FBME, glibenclamide low and high doses and the combination of the FBME and both doses of glibenclamide not only sustained the weight loss due to alloxan but also exhibited improvement in body weight, which may be due to improvement in glycemic control. Combination therapy was found to be more beneficial in long term treatment.

The hypoglycemic potency of the methanolic extract of bark of *Ficus glomerata* Roxb. may be attributed to the vital phytoconstituents contained, viz.- sterols, saponins, glycosides, glaunol, tannins and other polyphenolic compounds. The anti-oxidant and free radical scavenging properties of polyphenolic compounds of the methanolic extract of stem bark of the plant might be responsible for its antidiabetic activity.[18]

Glibenclamide, a 2nd generation sulfonylurea antidiabetic agent, lowers blood glucose acutely by stimulating the release of insulin from the pancreas. With chronic administration of glibenclamide in Type II diabetic patients the blood glucose lowering effect persists, but there is a gradual decline in the insulin secretory response to the drug in Type II diabetes.[19] Besides this special precautions are to be taken for administrating the glibenclamide in patients with decreased kidney function, liver function and with severe thyroid and adrenal gland problems.[20] In this regard the dosage of glibenclamide should be reduced to reduce the side effects in long-term treatment. The drug also has been claimed to have additive hypoglycemic effect with other antidiabetic drugs.[21]

The results observed suggest that the *Ficus glomerata* bark extract (FBME) when combined with glibenclamide enhances the hypoglycemic activity of the latter. The results of the study indicate the beneficial herb-drug interaction of combining FBME with glibenclamide and also the

combination could provide an opportunity to reduce the dose of glibenclamide for minimizing the adverse effects and achieving enhanced therapeutic effect. At the same time proper precaution and care should be taken as the combination therapy may pose the condition of severe hypoglycemia.

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Table 1. Effect of FBME and glibenclamide combinations on blood glucose levels in oral glucose tolerance test in normal rats

Groups	Blood glucose (mg/dl)			
	O min	30 min	60 min	90 min
Control	86.33±6.716	119.7±3.575	109.2±2.286	91.00±3.425
Gl-300 µg/kg	72.50±2.349	136.5±15.62	99.50±10.73	68.83*±5.294
Gl-600 µg/kg	89.67±6.464	122.3±1.745	100.7±4.709	71.33*±6.075
FBME 200mg/kg	70.33±2.472	156.7±13.26	113.2±7.867	89.67±6.103
FBME 200mg/kg + Gl-300 µg/kg	70.17±2.315	142.8±4.487	87.33*±2.348	59.50**±3.253
FBME 200mg/kg + Gl-600 µg/kg	65.33±1.726	136.5±1.544	102.8±1.740	79.67±2.108

FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide
Values are expressed as Mean ± SEM.;*p<0.01, **p<0.001

Table 2. Effect of FBME and glibenclamide combinations on blood glucose levels in acute antidiabetic study in alloxan-induced diabetic rats

Groups	Blood glucose (mg/dl)			
	Basal value (O h)	1 h	3 h	5 h
Control	78.33±2.171	78.67±2.525	79.83±2.227	79.67±2.486
Diabetic Control	335.8±2.725	346.0±2.887	352.5±3.631	361.7±3.602
Gl-300 µg/kg	307.0±6.658	238.0**±12.27	207.0**±6.748	179.2**±8.232
Gl-600 µg/kg	311.7±7.500	243.0**±9.825	208.3**±10.68	164.8**±9.782
FBME 200mg/kg	330.3±9.545	331.5**±10.62	312.8**±12.69	283.8**±14.45
FBME 200mg/kg + Gl-300 µg/kg	331.2±11.71	299.5**±24.74	187.3**±24.53	134.8±18.17
FBME 200mg/kg + Gl-600 µg/kg	328.3±10.16	313.7**±10.91	276.2*±10.66	191.3**±7.069

FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide
Values are expressed as Mean ± SEM.; *p<0.01, **p<0.001

Table 3. Effect of FBME and glibenclamide combinations on blood glucose levels in subacute anti-diabetic study in alloxan-induced diabetic rats

Groups	Blood glucose (mg/dl)			
	Basal value (O day)	7th day	14th day	21st day
Control	78.33±2.171	78.83±3.038	76.83±2.496	75.83±2.344
Diabetic Control	335.8±2.725	387.0±3.679	407.0±2.910	421.7±2.390
Gl-300 µg/kg	307.0±6.658	155.8***±7.922	126.7**±5.795	95.33*±5.200
Gl-600 µg/kg	311.7±7.500	139.3**±8.301	116.7*±7.969	87.00±5.247
FBME 200mg/kg	330.3±9.545	259.5***±12.89	197.2***±18.35	117.8***±9.799
FBME 200mg/kg + Gl-300 µg/kg	331.2±11.71	154.0***±10.65	111.5±6.864	82.67±4.667
FBME 200mg/kg + Gl-600 µg/kg	328.3±10.16	110.0±9.879	79.0±4.740	71.83±1.046

FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide
Values are expressed as Mean ± SEM.;*p<0.05, **p<0.01, ***p<0.001

Table 4. Effect of FBME and Glibenclamide combinations on body weight in alloxan diabetic rats

Groups	Body weight (in g)			
	Basal value (O day)	7th day	14th day	21st day
Control	**164.8±2.372**	**168.7±2.186**	**174.2±2.212**	**176.7±5.011**
Diabetic Control	**157.5±1.784**	**144.0±2.352**	**123.0±3.768**	**111.8±2.960**
Gl-300 µg/kg	**170.3±2.565**	**177.8±2.701**	**187.2±2.007**	**196.7**±2.201**
Gl-600 µg/kg	**162.0±2.352**	**171.7±2.974**	**181.0±3.924**	**194.3*±1.542**
FBME 200mg/kg	**185.0±4.282**	**191.2**±4.135**	**195.0*±4.782**	**199.5**±5.439**
FBME 200mg/kg + Gl-300 µg/kg	**166.0±4.163**	**170.5±4.023**	**175.5±3.704**	**178.8±3.637**
FBME 200mg/kg + Gl-600 µg/kg	**180.0±7.746**	**186.0*±5.704**	**188.7±6.059**	**195.2**±5.319**

FBME- *Ficus glomerata* Bark Methanolic Extract, Gl- Glibenclamide
Values are expressed as Mean ± SEM.;*p<0.05, **p<0.01.
Test drug treated groups were compared with control group (Group I)